

APPLICANT COPY

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/00, 15/12, C07K 14/00, 14/435		A1	(11) International Publication Number: WO 99/58660 (43) International Publication Date: 18 November 1999 (18.11.99)																																							
<p>(21) International Application Number: PCT/US99/09847</p> <p>(22) International Filing Date: 6 May 1999 (06.05.99)</p> <p>(30) Priority Data:</p> <table> <tbody> <tr><td>60/085,093</td><td>12 May 1998 (12.05.98)</td><td>US</td></tr> <tr><td>60/085,094</td><td>12 May 1998 (12.05.98)</td><td>US</td></tr> <tr><td>60/085,105</td><td>12 May 1998 (12.05.98)</td><td>US</td></tr> <tr><td>60/085,180</td><td>12 May 1998 (12.05.98)</td><td>US</td></tr> <tr><td>60/085,927</td><td>18 May 1998 (18.05.98)</td><td>US</td></tr> <tr><td>60/085,906</td><td>18 May 1998 (18.05.98)</td><td>US</td></tr> <tr><td>60/085,924</td><td>18 May 1998 (18.05.98)</td><td>US</td></tr> <tr><td>60/085,922</td><td>18 May 1998 (18.05.98)</td><td>US</td></tr> <tr><td>60/085,923</td><td>18 May 1998 (18.05.98)</td><td>US</td></tr> <tr><td>60/085,921</td><td>18 May 1998 (18.05.98)</td><td>US</td></tr> <tr><td>60/085,925</td><td>18 May 1998 (18.05.98)</td><td>US</td></tr> <tr><td>60/085,928</td><td>18 May 1998 (18.05.98)</td><td>US</td></tr> <tr><td>60/085,920</td><td>18 May 1998 (18.05.98)</td><td>US</td></tr> </tbody> </table>		60/085,093	12 May 1998 (12.05.98)	US	60/085,094	12 May 1998 (12.05.98)	US	60/085,105	12 May 1998 (12.05.98)	US	60/085,180	12 May 1998 (12.05.98)	US	60/085,927	18 May 1998 (18.05.98)	US	60/085,906	18 May 1998 (18.05.98)	US	60/085,924	18 May 1998 (18.05.98)	US	60/085,922	18 May 1998 (18.05.98)	US	60/085,923	18 May 1998 (18.05.98)	US	60/085,921	18 May 1998 (18.05.98)	US	60/085,925	18 May 1998 (18.05.98)	US	60/085,928	18 May 1998 (18.05.98)	US	60/085,920	18 May 1998 (18.05.98)	US	<p>(US). FLORENCE, Kimberly [US/US]; 12805 Atlantic Avenue, Rockville, MD 20851 (US). NI, Jian [CN/US]; 5502 Manorfield Road, Rockville, MD 20853 (US). ROSEN, Craig, A. [US/US]; 22400 Rolling Hill Road, Laytonsville, MD 20882 (US). CARTER, Kenneth, C. [US/US]; 11601 Brandy Hall Lane, North Potomac, MD 20878 (US). MOORE, Paul, A. [US/US]; 19005 Leatherbark Drive, Germantown, MD 20874 (US). OLSEN, Henrik, S. [DK/US]; 182 Kendrick Place #24, Gaithersburg, MD 20878 (US). SHI, Yang-gu [CN/US]; 437 West Side Drive, Gaithersburg, MD 20878 (US). YOUNG, Paul, E. [US/US]; 122 Beckwith Street, Gaithersburg, MD 20878 (US). WEI, Fing-Fei [CN/US]; 242 Gravatt Drive, Berkeley, CA 94705 (US). BREWER, Laurie, A. [US/US]; Apartment 115, 410 Van Dyke Street, St. Paul, MN 55119-4321 (US). SOPPET, Daniel, R. [US/US]; 15050 Stillfield Place, Centreville, VA 22020 (US). LAFLEUR, David, W. [US/US]; 3142 Quesada Street, N.W., Washington, DC 20015 (US). ENDRESS, Gregory, A. [US/US]; 9729 Clagett Farm Drive, Potomac, MD 20854 (US). EBNER, Reinhard [DE/US]; 9906 Shelburne Terrace #316, Gaithersburg, MD 20878 (US).</p> <p>(71) Applicant (for all designated States except US): HUMAN GENOME SCIENCES, INC. [US/US]; 9410 Key West Avenue, Rockville, MD 20850 (US).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): RUBEN, Steven, M. [US/US]; 18528 Heritage Hills Drive, Olney, MD 20832</p> <p>(74) Agents: BROOKES, A., Anders et al.; Human Genome Sciences, Inc., 9410 Key West Avenue, Rockville, MD 20850 (US).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p>	
60/085,093	12 May 1998 (12.05.98)	US																																								
60/085,094	12 May 1998 (12.05.98)	US																																								
60/085,105	12 May 1998 (12.05.98)	US																																								
60/085,180	12 May 1998 (12.05.98)	US																																								
60/085,927	18 May 1998 (18.05.98)	US																																								
60/085,906	18 May 1998 (18.05.98)	US																																								
60/085,924	18 May 1998 (18.05.98)	US																																								
60/085,922	18 May 1998 (18.05.98)	US																																								
60/085,923	18 May 1998 (18.05.98)	US																																								
60/085,921	18 May 1998 (18.05.98)	US																																								
60/085,925	18 May 1998 (18.05.98)	US																																								
60/085,928	18 May 1998 (18.05.98)	US																																								
60/085,920	18 May 1998 (18.05.98)	US																																								
<p>(54) Title: 97 HUMAN SECRETED PROTEINS</p> <p>(57) Abstract</p> <p>The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.</p>																																										

260

265

<210> 138
<211> 434
<212> PRT
<213> Homo sapiens

<400> 138
Met Ala Pro Glu Gly Leu Val Pro Ala Val Leu Trp Gly Leu Ser Leu
1 5 10 15
Phe Leu Asn Leu Pro Gly Pro Ile Trp Leu Gln Pro Ser Pro Pro Pro
20 25 30
Gln Ser Ser Pro Pro Pro Gln Pro His Pro Cys His Thr Cys Arg Gly
35 40 45
Leu Val Asp Ser Phe Asn Lys Gly Leu Glu Arg Thr Ile Arg Asp Asn
50 55 60
Phe Gly Gly Asn Thr Ala Trp Glu Glu Asn Leu Ser Lys Tyr
65 70 75 80
Lys Asp Ser Glu Thr Arg Leu Val Glu Val Leu Glu Gly Val Cys Ser
85 90 95
Lys Ser Asp Phe Glu Cys His Arg Leu Leu Glu Leu Ser Glu Glu Leu
100 105 110
Val Glu Ser Trp Trp Phe His Lys Gln Gln Glu Ala Pro Asp Leu Phe
115 120 125
Gln Trp Leu Cys Ser Asp Ser Leu Lys Leu Cys Cys Pro Ala Gly Thr
130 135 140
Phe Gly Pro Ser Cys Leu Pro Cys Pro Gly Gly Thr Glu Arg Pro Cys
145 150 155 160
Gly Gly Tyr Gly Gln Cys Glu Gly Glu Gly Thr Arg Gly Ser Gly
165 170 175
His Cys Asp Cys Gln Ala Gly Tyr Gly Glu Ala Cys Gly Gln Cys
180 185 190
Gly Leu Gly Tyr Phe Glu Ala Glu Arg Asn Ala Ser His Leu Val Cys
195 200 205
Ser Ala Cys Phe Gly Pro Cys Ala Arg Cys Ser Gly Pro Glu Glu Ser
210 215 220
Asn Cys Leu Gln Cys Lys Lys Gly Trp Ala Leu His His Leu Lys Cys
225 230 235 240
Val Asp Ile Asp Glu Cys Gly Thr Glu Gly Ala Asn Cys Gly Ala Asp
245 250 255
Gln Phe Cys Val Asn Thr Glu Gly Ser Tyr Glu Cys Arg Asp Cys Ala
260 265 270
Lys Ala Cys Leu Gly Cys Met Gly Ala Gly Pro Gly Arg Cys Lys Lys
275 280 285
Cys Ser Pro Gly Tyr Gln Gln Val Gly Ser Lys Cys Leu Asp Val Asp

290	295	300
Glu Cys Glu Thr Glu Val Cys Pro Gly Glu Asn Lys Gln Cys Glu Asn		
305	310	315
Thr Glu Gly Gly Tyr Arg Cys Ile Cys Ala Glu Gly Tyr Lys Gln Met		
325	330	335
Glu Gly Ile Cys Val Lys Glu Gln Ile Pro Gly Ala Phe Pro Ile Leu		
340	345	350
Thr Asp Leu Thr Pro Glu Thr Thr Arg Arg Trp Lys Leu Gly Ser His		
355	360	365
Pro His Ser Thr Tyr Val Lys Met Lys Met Gln Arg Asp Glu Ala Thr		
370	375	380
Phe Pro Gly Leu Tyr Gly Lys Gln Val Ala Lys Leu Gly Ser Gln Ser		
385	390	395
Arg Gln Ser Asp Arg Gly Thr Arg Leu Ile His Val Ile Asn Ala Leu		
405	410	415
Pro Pro Thr Cys Pro Pro Gln Lys Lys Lys Lys Lys Lys Lys Lys Gly		
420	425	430

Gly Arg

<210> 139
<211> 237
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (55)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (237)
<223> Xaa equals stop translation

<400> 139
Met Ile Ser Leu Pro Gly Pro Leu Val Thr Asn Leu Leu Arg Phe Leu
1 5 10 15

Phe Leu Gly Leu Ser Ala Leu Ala Pro Pro Ser Arg Ala Gln Leu Gln
20 25 30

Leu His Leu Pro Ala Asn Arg Leu Gln Ala Val Glu Gly Gly Glu Val
35 40 45

Val Leu Pro Ala Trp Tyr Xaa Leu His Gly Glu Val Ser Ser Ser Gln
50 55 60

Pro Trp Glu Val Pro Phe Val Met Trp Phe Phe Lys Gln Lys Glu Lys
65 70 75 80

Glu Asp Gln Val Leu Ser Tyr Ile Asn Gly Val Thr Thr Ser Lys Pro
85 90 95

- invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" 5 includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the 10 carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form.

Furthermore, any combination of the above amino and carboxy terminus deletions are 15 preferred. Similarly, polynucleotide fragments encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic 20 regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotide 25 fragments encoding these domains are also contemplated.

Other preferred fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable 30 activity.

Epitopes & Antibodies

In the present invention, "epitopes" refer to polypeptide fragments having antigenic or immunogenic activity in an animal, especially in a human. A preferred embodiment of the present invention relates to a polypeptide fragment comprising an epitope, as well as the polynucleotide encoding this fragment. A region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." In contrast, an "immunogenic epitope" is defined as a part of a protein that elicits an antibody response. (See, for instance, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983).)

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,211.)

In the present invention, antigenic epitopes preferably contain a sequence of at least seven, more preferably at least nine, and most preferably between about 15 to about 30 amino acids. Antigenic epitopes are useful to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe, J. G. et al., Science 219:660-666 (1983).)

Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow, M. et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle, F. J. et al., J. Gen. Virol. 66:2347-2354 (1985).) A preferred immunogenic epitope includes the secreted protein. The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting.)

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')₂ fragments) which are capable of specifically binding to protein. Fab and F(ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. (Wahl et al., J. Nucl. Med. 24:316-325 (1983).) Thus, these

fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimeric, single chain, and humanized antibodies.

5 Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second 10 protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous 15 functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the 20 polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

25 Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4- 30 polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the